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Published in:
Tetrahedron

DOI:
10.1016/S0040-4020(03)00461-7

Publication date:
2003

Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 3925–3935

TETRAHEDRON

Peptide-functionalized polyphenylene dendrimers

Andreas Herrmann, Gueorgui Mihov, Guido W. M. Vandermeulen, Harm-Anton Klok and Klaus Müllen*

Max Planck Institute for Polymer Research, Ackermannweg 10, D-55128 Mainz, Germany

Received 26 September 2002; revised 14 November 2002; accepted 10 January 2003

Abstract—This contribution describes the synthesis of polyphenylene dendrimers that are functionalized with up to 16 lysine residues or substituted with short peptide sequences composed of 5 lysine or glutamic acid repeats and a C- or N-terminal cysteine residue. Polyphenylene dendrimers were prepared via a sequence of Diels–Alder cycloaddition and deprotection reactions from cyclopentadienone building blocks. Single amino acids could be introduced on the periphery of the dendrimers by using amino acid substituted cyclopentadienones in the last Diels–Alder addition reaction. Alternatively, peptide sequences were attached via a chemoselective reaction, which involved the addition of the sulfhydryl group of a cysteine residue of an oligopeptide to a maleimide moiety present on the surface of the dendrimer. These amino acid and peptide functionalized dendrimers may be of interest as model compounds to study DNA complexation and condensation or as building blocks for the preparation of novel supramolecular architectures via layer-by-layer self-assembly.

1. Introduction

In contrast to conventional polymers, dendrimers are highly branched, monodisperse macromolecules with a high number of surface functionalities.[1–3] Dendrimers differ from polymers with respect to their methods of preparation.[4,5] They are synthesized either by a convergent or divergent approach, both implicating the generation of a core molecule and an iterative procedure for the creation of the dendrons. The structural perfection of dendrimers is a prerequisite for clinical applications. Recent advances in this respect, concerning therapeutic and diagnostic approaches, have been reviewed.[6] For diagnostic purposes we have recently created a well defined dendrimer, containing three chromophores and one biotin moiety at the periphery.[7] The perfect structural and spatial definition of functions results from the use of a shape persistent dendritic polyphenylene scaffold, consisting of pentaphenylbenzene units and a rigid tetrahedral core molecule. In previous work we have demonstrated that, by applying this dendrimer type, we cannot only place a certain number of different functionalities into a defined volume element, which also holds true for other dendrimer-types, but can even control their orientation.[8–10]

In this publication, we describe the synthesis of several new polyphenylene dendrimers that contain a large number of lysine residues on their periphery or which are functionalized with short peptide sequences. These peptide sequences are composed of five lysine or glutamic acid residues and a C- or N-terminal cysteine moiety. Our interest in polyphenylene–peptide dendrimers is twofold. First of all, the cationic lysine functionalized dendrimers may serve as model compounds to study DNA complexation and condensation.[11] Such model compounds may help to further understand the pathway of gene transfection. Second, both the cationic lysine and anionic glutamic acid functionalized dendrimers are interesting as building blocks for the electrostatic layer-by-layer self-assembly of novel supramolecular architectures.[12] Layer-by-layer self-assembly may involve either alternating deposition of oppositely charged dendrimers or of dendrimers and linear polyelectrolytes of opposite charge. The shape persistence of the polyphenylene dendrimer scaffold may allow the preparation of supramolecular assemblies possessing defined nanocavities.

2. Results and discussion

The divergent synthesis of polyphenylene dendrimers, which will be described here, starts from multi ethyne-substituted core molecules, the geometry of which determines the overall shape of the resulting cascade molecules.[13] The generation of each dendritic layer is based on an iterative process including a Diels–Alder reaction and subsequent deprotection of ethynyl functions. The branching agents that are employed are bis silylethyne substituted tetracyclones, which can act both as diene and, after removal of the silyl protective groups from the ethyne groups, as dienophiles. In the [4+2]-cycloaddition between

Keywords: dendrimers; lysine residues; hexapeptide.

* Corresponding author. Tel.: +49-6131-379-151; fax: +49-6131-379-350; e-mail: muellen@mpip-mainz.mpg.de

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doi:10.1016/S0040-4020(03)00461-7

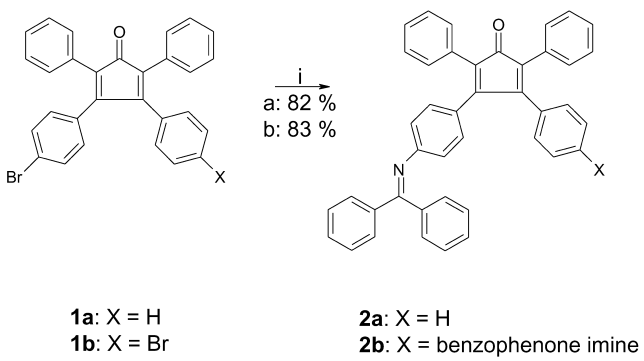

Figure 1. Synthesis of functionalized cyclopentadienones. (i) Benzophenone imine, BINAP, Cs_2CO_3, $Pd(dba)_2$, toluene.

the cyclopentadienone and a free ethyne group of the core molecule or the preceding dendrimer generation, a tetraphenylbenzene unit is formed with the extrusion of carbon monoxide.[14–17]

Three routes for the functionalization of polyphenylene dendrimers have been reported.[18] First, functional groups can be introduced by electrophilic aromatic substitution. This leads to a statistical distribution of functional groups on the periphery of the dendrimer, which is not desired within the context of this contribution. A second strategy, which allows the controlled introduction of sensitive functionalities like biological molecules, starts from dendrimers which are already functionalized. The corresponding functional groups on the rim of the dendrimer are generated by a functionalized cyclopentadienone in a Diels–Alder reaction at elevated temperatures. Such cyclopentadienones do not carry any protected ethyne groups like the branching reagent, but act as terminating reagents bearing only functional groups. These functional groups are then converted into the desired functions in a polymer-analogous reaction on the dendritic scaffold. A third approach to introduce multiple functional groups involves the use of cyclopentadienones that are already substituted with the appropriate functional group.[19,20] This approach requires the possibility of incorporating the respective function into the cyclopentadienone and sufficient stability of the functional groups against the Diels–Alder conditions, i.e. elevated temperatures. An advantage of this approach is that it allows quantitative introduction of the functional groups due to the high yield of the [4+2]-cycloaddition. This paper reports the use of the latter two approaches to introduce lysine moieties and short amino acid sequences based on lysine and glutamic acid onto the surface of polyphenylene dendrimers.

2.1. Synthesis of functionalized dendrimers via polymer-analogous reactions

As amino groups offer a wide range of possibilities for further functionalization, we chose these as surface functionalities. To incorporate amino groups at the rim of the dendrimer the corresponding function must be introduced into the cyclopentadienone. The synthesis of the functionalized cyclopentadienones is outlined in Figure 1 and starts with monobromo- (**1a**) and dibromotetracyclopentadienone (**1b**). In the first reaction step, the halogen atoms are replaced in a palladium-catalyzed aryl-*N*-coupling by benzophenone imine, which is an elegant way for the introduction of an amino group as a synthetic equivalent of ammonia.[21–24] An additional advantage of benzophenone imine, in contrast to a non-protected amine,

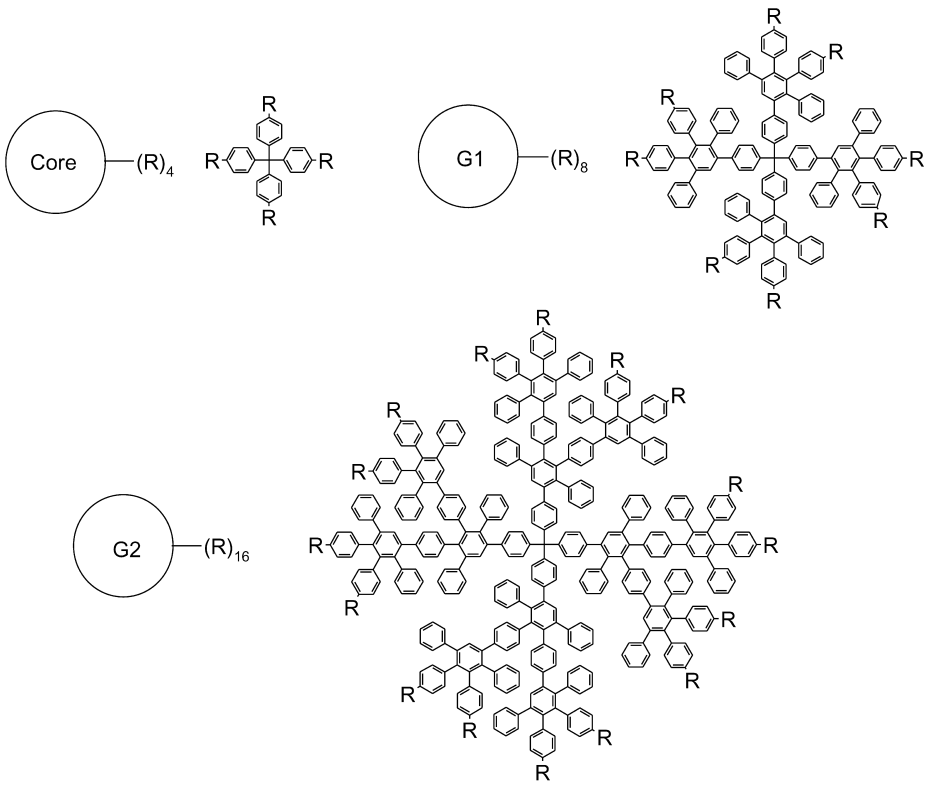

Figure 2. Abbreviations.

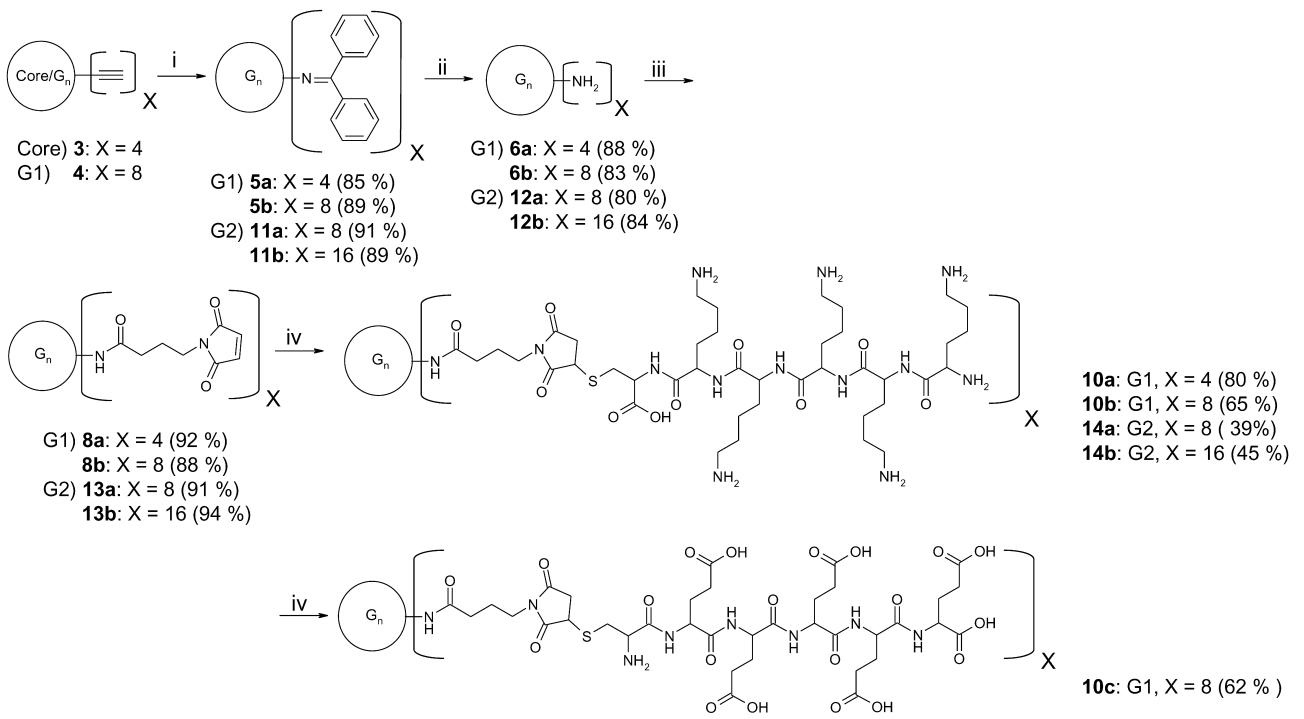

Figure 3. Synthesis of oligolysine-functionalized G1/G2-dendrimers. (i) **2a** or **2b**, *o*-xylene, 145°C (G1) or tetraethylene glycol/diphenyl ether, 170°C (G2). (ii) HCl, rt, THF. (iii) **7**, triethylamine, DMF, rt. (iv) **9**, DMF, rt.

is its thermostability and therefore its suitability as a reagent in the cycloaddition. The synthesis of both monofunctional (**2a**)[25] and difunctional (**2b**)[26] cyclopentadienones, offers the possibility to control the surface concentration of functional groups.

For the sake of clarity, the terminology in Figure 2 will be used to represent the central dendritic polyphenylene units in the following paragraphs. Employing the terminating reagents **2a**,**b** in a Diels–Alder reaction with either the tetrahedral core molecule **3** or the octaethynyl G1-dendrimer **4** yielded the benzophenone imine-functionalized dendrimers **5a**,**b** and **11a**,**b** (Fig. 3). The synthesis of the starting materials **3** and **4** has been described previously.[27] For the generation of the G1-dendrimers **5a**,**b**, *o*-xylene was applied as a solvent at 145°C. The formation of the next higher generation dendrimers **11a**,**b** required temperatures of 170°C and a solvent mixture of tetraethyleneglycol and biphenylether. Subsequently, complete removal of the protective groups was achieved with HCl in THF at rt. The resulting dendrimers **6a**,**b** and **12a**,**b** possess 4, 8 and 16 amino groups on their surface.

The attachment of a defined number of oligopeptide sequences onto polyphenylene dendrimers involved the addition of the sulfhydryl group of a terminal cysteine residue to a maleimide function on the periphery of the dendrimer. It was found that 4-maleimide-butyric acid chloride (**7**), which was prepared according to a literature procedure,[28] reacted quantitatively with the amino functions of the dendrimers **6a**,**b** and **12a**,**b** to yield the maleimide-functionalized structures **8a**,**b** and **13a**,**b** (Fig. 3). In a subsequent step, the thiol group of peptides **9a**,**b** was reacted with the maleimide functions of the dendritic scaffold. Hexapeptide **9a** is composed of a C-terminal cysteine and five lysine residues, and **9b** is composed of a N-terminal cysteine and five glutamic acid residues. These peptides were prepared via solid-phase peptide synthesis using Fmoc protective group chemistry.[29] For the coupling reaction with

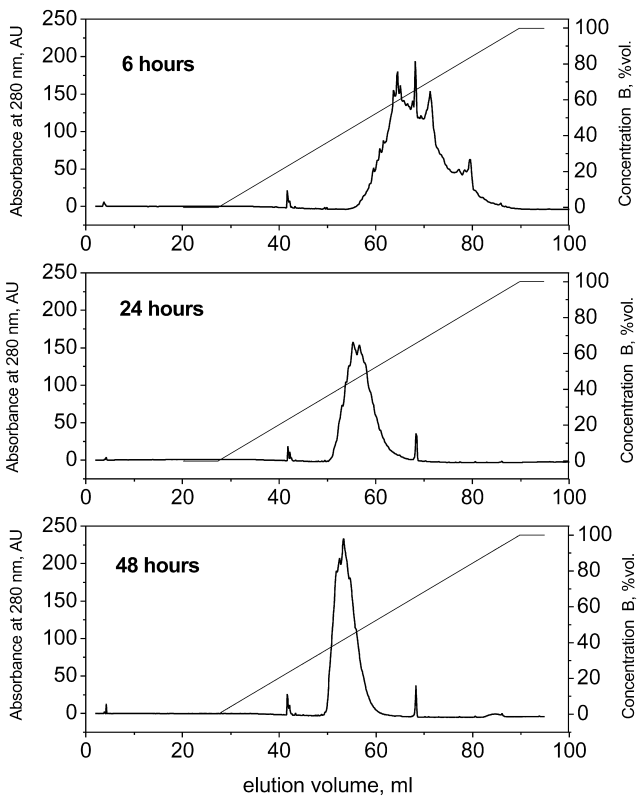

Figure 4. HPLC-monitoring of the coupling of hexapeptide **9** to the maleimide-functionalized dendrimer **8b** to form dendrimer **10b**.

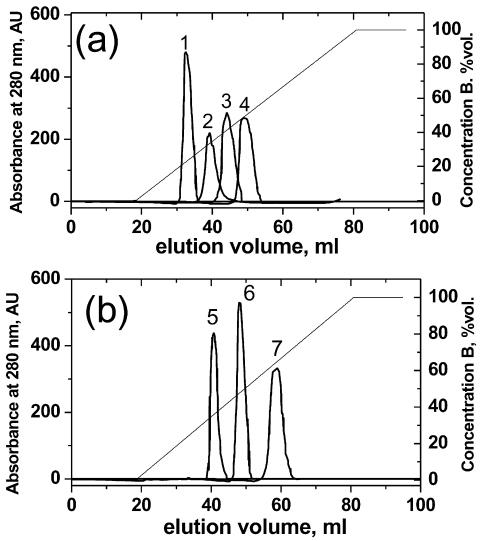

Figure 5. RP-HPLC chromatograms of all compounds. (a) Compounds **10a** (peak 1), **10b** (peak 2), **14b** (peak 3), and **14a** (peak 4), prepared by polymer-analogous reaction. (b) Compounds **21b** (peak 5), **21a** (peak 6), and **19** (peak 7), prepared by functionalized cyclopentadienones.

the dendrimer, **9a**,**b** were applied in a three-fold excess with respect to the dendrimers. The reaction was carried out under mild conditions in DMF, which was removed in the work-up by dialysis against water. The addition of hexapeptide **9a** to **8b** was followed by reversed-phase HPLC. Samples from the reaction mixture were analyzed at 6, 24 and 48 h after the start of the reaction (Fig. 4). The total reaction time was 72 h. Two observations can be made. First, there is a shift of the major peak from higher to lower elution volumes during the process of reaction, i.e. to a solvent mixture of higher water content. The compound becomes more hydrophilic with increasing reaction time, which can be explained by the attachment of an increasing number of (hydrophilic) peptide chains. Second, the major peak becomes sharper and more symmetrical at longer reaction times, indicating the decrease of polydispersity. In Figure 5, chromatograms of all compounds are shown to give a single peak and document their purity and monodispersity.

Figure 6 shows a comparison of the ^{1}H NMR spectra of dendrimer **8b** and the peptide-decorated dendrimer **10b**, the latter being separated from the reaction mixture by dialysis and purified by RP-HPLC. The ^{1}H NMR spectrum of **8b** shows the aromatic signals of the dendritic scaffold in the region of 6.4 to 7.4 ppm. The protons of the maleimide linker appear as singlets at 1.71, 2.18 and 3.38 ppm as well as a doublet at 6.82 ppm, which overlaps with the aromatic protons of the polyphenylene signals. When the hexapeptide **9a** is attached and dendrimer **10b** is generated, the doublet for the maleimide at low field disappears to form two singlets. One appears at 3.82 ppm and the other coincides with the water peak at 3.25 ppm and is therefore not detectable. The remaining aliphatic signals of the maleimide linker as well as all signals of the peptide are found in the ^{1}H NMR spectrum of **10b**. For the exact assignment of the peptide signals see Figure 6. The ^{1}H NMR spectrum of **10b** supports the proposed structure and also proves the purity of compound **10b**. Similar spectra are obtained for dendrimer **10a** which show the defect-free attachment of peptide **9a**. It is important to mention, that with the exception of the last step of the reaction sequence, the work-up of all dendritic intermediates is realized simply by precipitation from methanol and filtration. Consequently, all reactions described in this paragraph give yields higher than 80%, thus illustrating the efficiency of this route of functionalization. The obtained G1-dendrimers **10a**,**b** contain 20 and 40 lysine residues on the surface, respectively. The next higher generation dendrimer is decorated with 40 and 80 lysines corresponding to **14a**,**b**, respectively. For the G2-dendrimers **14a**,**b**, there are therefore 48 and 96 primary amino groups on the surface. The purity of **14a**,**b** was also checked with ^{1}H NMR (spectra not shown) and RP-HPLC (Fig. 5). It was not possible to obtain mass spectra.

2.2. Synthesis of lysine-functionalized dendrimers from amino acid substituted cyclopentadienones

The synthesis of the lysine-functionalized cyclopentadienones started from the benzophenone imine tetraphenylcyclopentadienone derivatives **2a**,**b**, which were transformed into the corresponding amino compounds **15a**,**b** by the same method as applied for the dendritic structures **6a**,**b** and **12a**,**b** (Fig. 7). For the next step, a thermostable protective group for the amino acid must be selected. Among commercially available bisamino-protected derivatives of lysine, N^{α},N^{ε}-di(benzyloxycarbonyl)-L-lysine (**16**) is thermally very stable. Hence, **16** was reacted with **15a**,**b**, with EDC as a coupling reagent in DMF, to obtain the *Z*-protected lysine-functionalized cyclopentadienones **17a**,**b** (Fig. 7). After column chromatography, cyclopentadienones **17a**,**b** were obtained in 75 and 45% yield, respectively. Both compounds show a single peak in their mass spectrum with a mass of 796 and 1208 g/mol respectively, which agrees with the calculated molecular weight. These compounds serve as terminating reagents in the Diels–Alder reaction introducing one or two amino acid functions per free ethyne group, respectively.

The cycloaddition of the cyclopentadienones **17a**,**b** with the tetrahedral core molecule **3** and the octaethyne-functionalized G1-dendrimer **4** was carried out analogously to the preparation of **11a**,**b**, using the higher boiling solvent mixture at 170°C (Fig. 8). The corresponding dendrimers **18** and **20a**,**b** were obtained after precipitation in methanol without any cleavage of the protective groups. *Z*-groups were removed by applying a cleavage mixture consisting of HBr in ice-acetic acid to yield the dendrimers **19** and **21a**,**b**.[30] Purification was achieved by precipitation from diethylether. Comparison of the ^{1}H NMR spectra of **18** and **19**, which are shown in Figure 9, supports the complete removal of all *Z*-groups. The ^{1}H NMR spectrum of **18** exhibits the signals of the aromatic protons of the dendritic scaffold and of the *Z*-groups at 6.6 to 7.6 ppm. The signals of the lysines are detected at 4.12, 3.13, 1.88, 1.49 and 1.39 ppm, whereas the aliphatic benzylic protons of the protective groups show up at 5.03 ppm. The ^{1}H NMR spectrum of **19** is nearly identical with the exception that all benzylic protons at 7.35 and 5.03 ppm are absent. Furthermore, the relative intensities of the aromatic and aliphatic protons in both spectra perfectly match the

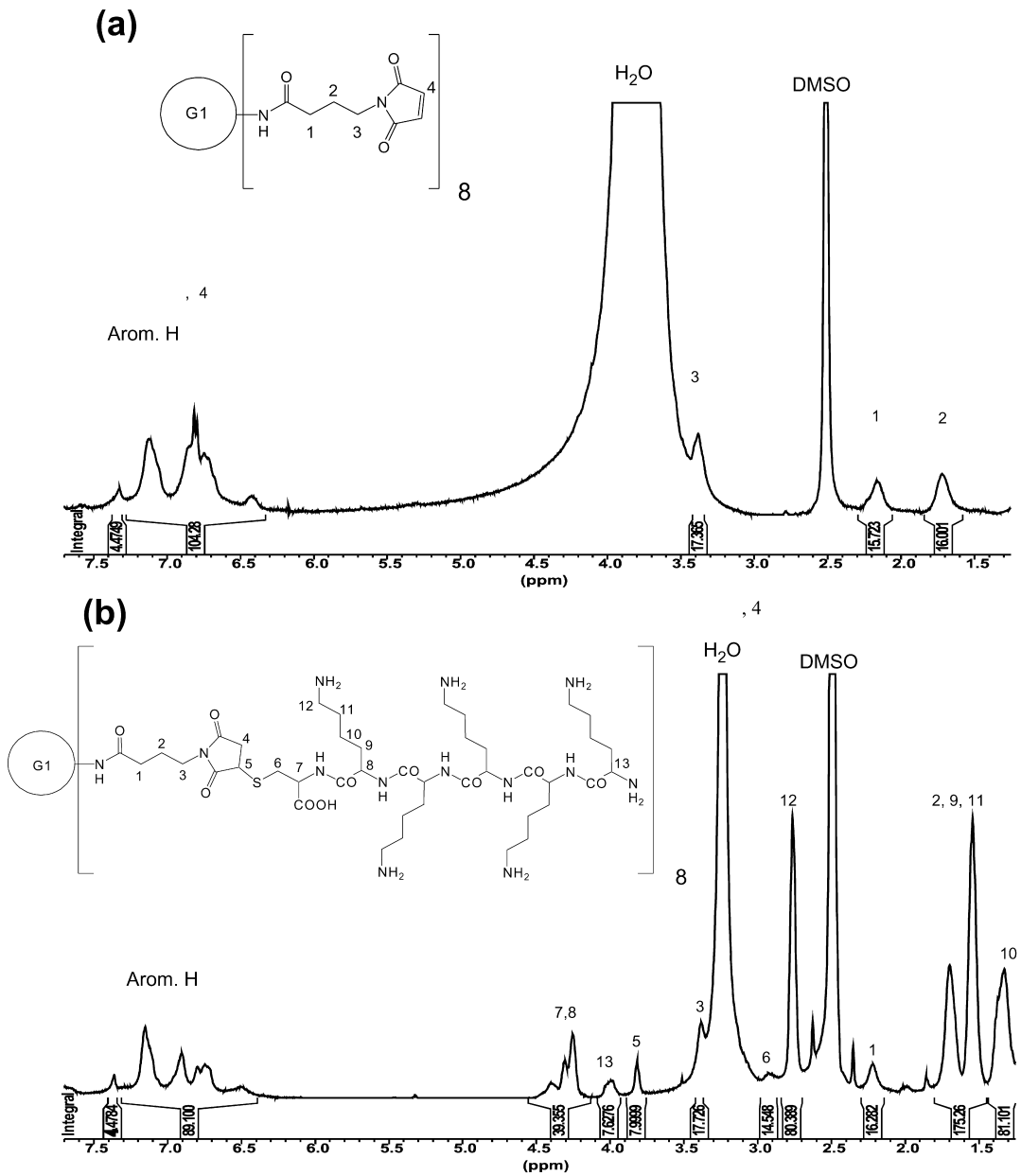

Figure 6. 500 MHz ^{1}H NMR-spectra (DMSO-d^6) of (a) **8b** and (b) **10b**.

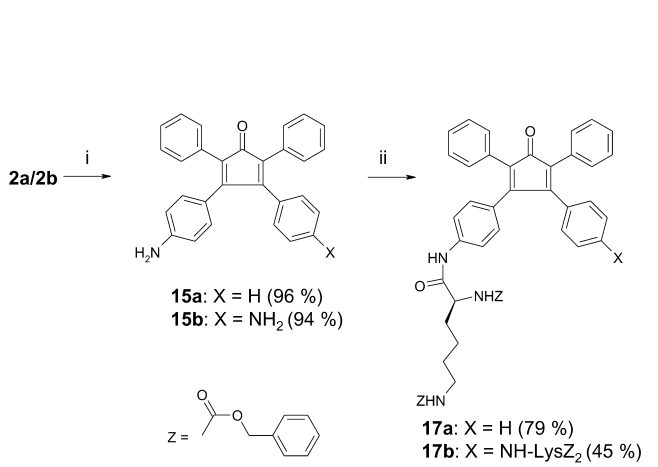

Figure 7. Synthesis of lysine-functionalized cyclopentadienones. (i) HCl, rt, THF; (ii) **16**, EDC, DMF, rt.

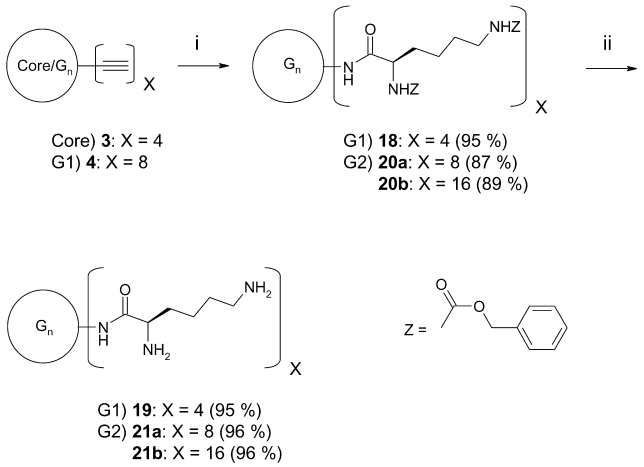

Figure 8. Synthesis of lysine-substituted G1/G2-dendrimers. (i) **17a** or **17b**, tetraethylene glycol/diphenyl ether, 170°C; (ii) HBr, HOAc, rt.

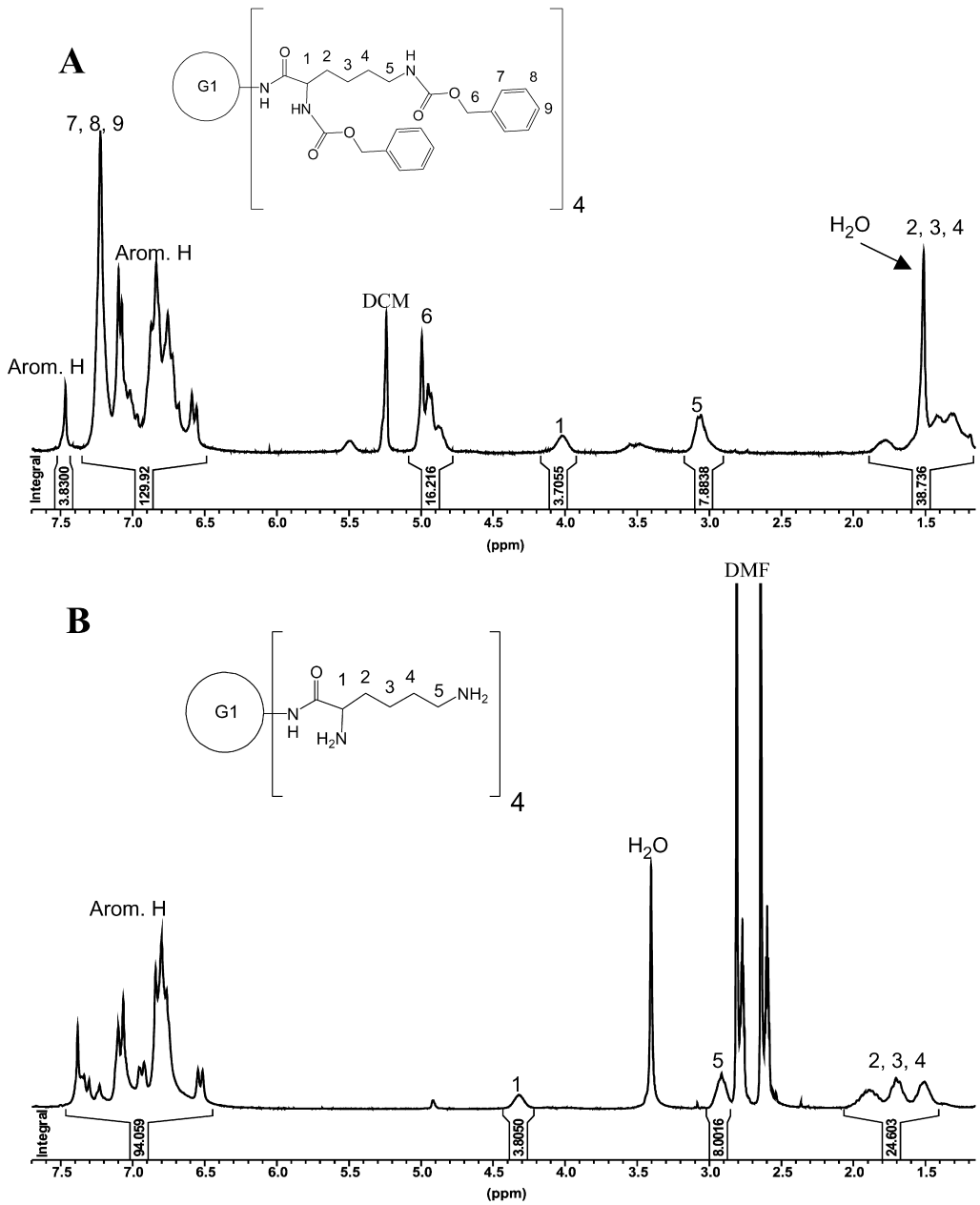

Figure 9. 300 MHz ^{1}H NMR-spectra of (a) **18** (CD_2Cl_2) and (b) **19** (DMF d^6).

calculated values. The complete removal of *Z*-groups is also shown in the MALDI-TOF mass spectra of **18** and **19** (Fig. 10). All yields for the cycloaddition and the deprotection are higher than 90%. The G1-dendrimer **19** possesses four lysine residues on the surface, whereas the G2-dendrimers **21a**,**b** bear 8 and 16 lysine moieties on the surface, respectively. Again expressed in terms of primary amine groups this equals 16 and 32 for the respective structures. The purity of **21a**,**b** was checked by ^{1}H NMR (spectra not shown) and RP-HPLC (Fig. 5). It was not possible to obtain mass spectra for these compounds.

Further support for the attachment of the cationic moieties to the surface comes from the change in solubility properties of these materials. As the surface density of lysine moieties increases when going from structures **19** and **21a**,**b** to structures **10a**,**b** and **14a**,**b**, the nature of the end groups influences the properties of the whole dendrimer more strongly. All polyphenylene dendrimers with free lysine groups on the surface are nicely soluble in DMSO and DMF. In contrast, when solubilization in water is desired, structures **19** and **21a**,**b** need to be predissolved in a very small amount of DMF or DMSO, whereas structures **10a**,**b** and **14a**,**b** are directly soluble in water. When no lysine residues are attached to the surface the polyphenylene dendrimers do not dissolve in water at all.

Summarizing, the two synthetic approaches give peptide-functionalized dendrimers in high yields and purity. Both routes provide oligolysine–dendrimers that behave in accord with the surface density of lysine residues and primary amine groups.

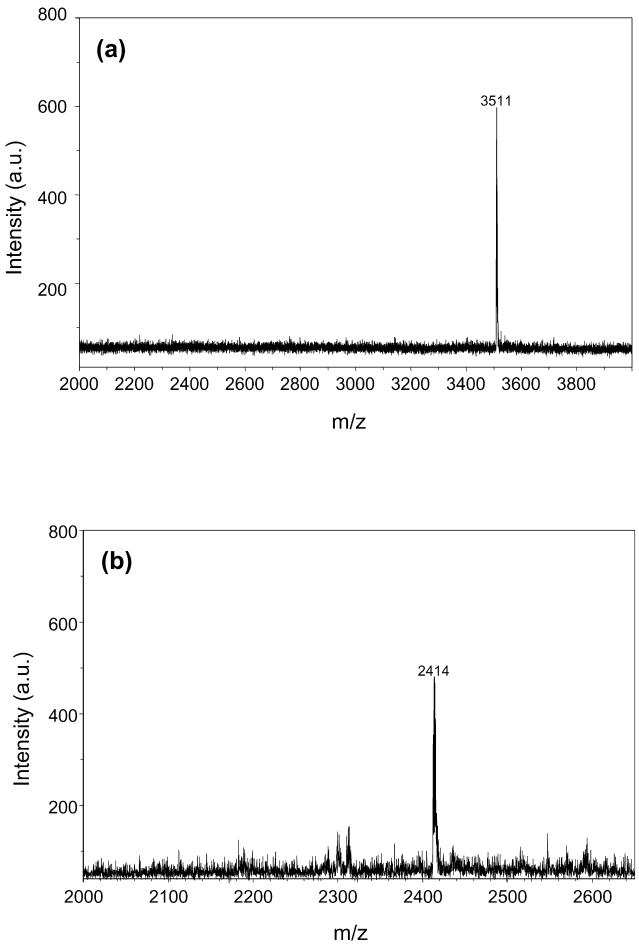

Figure 10. MALDI-TOF mass spectra of (a) **18** (m/z=3511 g/mol (M(Na+)), calcd MW 3488.26 g/mol) and (b) **19** (m/z=2414 g/mol (M+), calcd MW 2415.17 g/mol).

3. Visualization/simulation

The structures of the tetrahedral core and dendrons of the first and second generation were optimized separately using the MM2 (MM+) force field, as implemented in HyperChem 5.1 (Hypercube Inc.). Subsequently, optimization of the polyphenylene dendritic scaffold was performed by combining four first-generation dendrons and four second-generation dendrons with the core molecule and minimizing both systems. The obtained 'naked' scaffolds resemble the structures obtained by a more profound modeling approach of these structures very closely.[31] Only for the G1-dendritic framework was a global minimum obtained. Due to the fairly flat hypersurface obtained for the second-generation dendritic scaffold, the three-dimensional structure represents one out of several possible local minima. In the last step of our calculations to obtain the structures **10a**,**b**, **14a**,**b**, **19** and **21a**,**b** we attached the minimized lysine residue as well as the hexapeptide onto the G1- and the G2-dendrimer and performed a minimization of the entire hybrid structures. The peptide building blocks have been optimized by applying the amber force field, which proved to be suitable for such structures.

Table 1. Diameters of the dendrimers as determined from molecular modelling

Dendrimer	**10a**	**10b**	**14a**	**14b**	**19**	**21a**	**21b**
Diameter (nm)	4.0	4.0	6.0	6.0	3.0	4.5	4.5

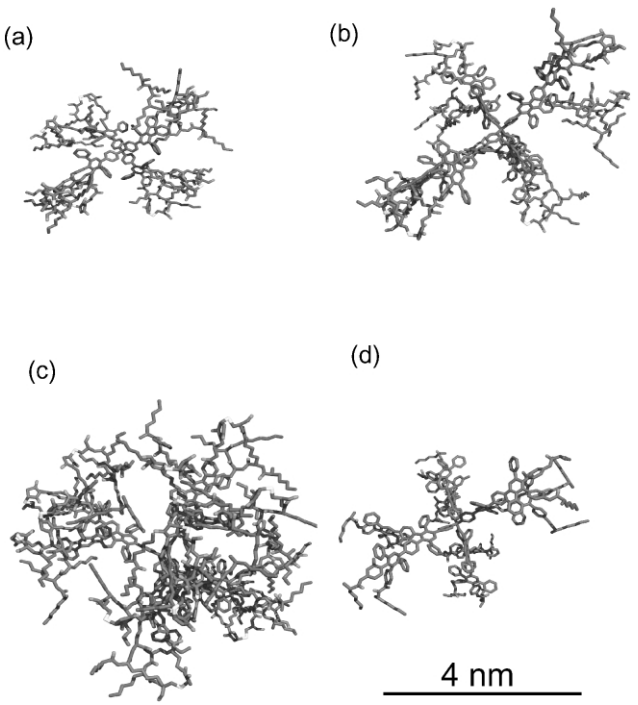

Figure 11. Molecular simulation of (a) **10b**, (b) **14a**, (c) **14b** and (d) **21b**.

The diameters of all molecules can be determined from the optimized three-dimensional structures, and are summarized in Table 1. The smallest compound with a diameter of 3 nm is the G1-dendrimer **19** bearing four lysine on the surface. The largest hybrid structures, exhibiting a diameter of 6 nm, are the G2-dendrimers **14a**,**b**, functionalized with the hexapeptide **9**. From Table 1, as well as from the 3D-structures in Figure 11, it can also be concluded that G1-dendrimer **10b**, containing a shell of the hexapeptide **9**, exhibits nearly the same size as the dendrimer **21b**, which is of the second generation, functionalized only with a shell of one amino acid. According to the simulation (Fig. 11), the lysine moieties are situated exclusively on the surface, where their spatial arrangement is well-defined. Comparing structures **14a**,**b**, it is clearly visible that in the case of **14b**, a dense, and in the case of **14a**, an open structure is formed.

4. Conclusions

We have presented two routes for the functionalization of shape persistent polyphenylene dendrimers with a large number of amino acids or oligopeptide sequences on the surface. These routes are essentially complementary with respect to the chemistry used for the preparation of the biofunctional dendrimers, but result in structurally similar molecules. The synthesis of the mono- and bis-lysine functionalized cyclopentadienones **17a**,**b** allows the construction of G1- and G2-dendrimers with low surface densities of amino acids. In the resulting structures the lysine residues are directly attached to the polyphenylene scaffold. Functionalization via a polymer-analogous

reaction, in contrast, yields first- and second-generation dendrimers with a high surface loading of lysine residues. In these structures, coupling was achieved by the addition of a terminal cysteine residue of a lysine or glutamic acid based hexapeptide to a maleimide group on the rim of the dendrimers. Important characteristics of the latter concept are the use of unprotected peptides, mild reaction conditions and the great flexibility for the attachment of different sequences. Currently, we are exploring these shape-persistent peptide functionalized dendrimers as model compounds for DNA complexation and condensation and are investigating their potential as building blocks for the electrostatic layer-by-layer self-assembly of ultrathin nanostructured supramolecular films.

5. Experimental

5.1. General information

Unless stated, all solvents and reagents are commercially available and were used as received. Compound **4** was synthesized according to a published procedure.[20] ^{1}H NMR spectra were recorded on Bruker DRX 500 (500 MHz) and Bruker AMX 250 (250 MHz) spectrometers. Spectra were referenced to the residual proton signal of the deuterated solvent. Molecular weights were determined with matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) or field desorption (FD) mass spectroscopy. MALDI-TOF spectra were recorded on a Bruker MALDI-TOF mass spectrometer. Peptide synthesis was carried out on an Applied Biosystems peptide synthesizer Model 433A. The amino acids were free acids and coupling was facilitated by the use of *O*-benzotriazol-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and *N*-hydroxybenzotriazole (HOBt). Purity was checked using analytical reversed-phase high pressure liquid chromatography (RP-HPLC, AKTA-Purifier, Amersham-Pharmacia Biotech). The column used was a reversed-phase C8 column (Sephasil peptide C8, volume 4.155 mL, 5 μm particle size). The lysine-dendrimers were eluted with a linear AB gradient from A to B over 61 min, with A consisting of a mixture of 98% water/2% acetonitrile (plus 0.065% TFA) and B of 100% acetonitrile (plus 0.05% TFA) in the case of **10a**,**b** and **14b**,**a**. The flow rate was 0.45 mL/min. Compounds **21a**,**b** and **19** were eluted with a linear AB gradient from A to B over 61 min, with A consisting of a mixture of 98% water/2% isopropanol (plus 0.1% TFA) and B of 100% isopropanol (plus 0.1% TFA). The flow rate was 1.0 mL/min at a temperature of 45°C. For the glutamic acid-dendrimer **10c** a Resource RPC with a volume of 3 mL was used. Compound **10c** was eluted with A consisting of a mixture of 98% water/2% acetonitrile (plus 0.03% Et_3N) and B of 100% acetonitrile (plus 0.03% Et_3N). The flow rate was 2 mL/min.

5.2. Procedures

5.2.1. Compound 2a. 3-(4-Bromophenyl)-2,4,5-triphenylcyclopenta-2,4-dien-1-one (**1**) (4.5 g, 9.71 mmol), BINAP (0.720 g, 1.15 mmol), tris(dibenzylideneacetone)-dipalladium(0) (0.356 g, 0.39 mmol), and $CsCO_3$ (20.94 g, 0.088 mol) were mixed in a 500 mL flask. The system was flushed with argon and freshly distilled toluene (250 mL) was added. Subsequently, benzophenone imine (10 mL, 0.055 mol) was added dropwise. The reaction mixture was left at 80°C for 4 days. Next, it was cooled down to rt and the toluene was evaporated. The residue was dissolved in tetrahydrofuran and precipitated in a mixture of methanol/water (7/1) to give **2a** as a brown powder. Yield: 4.5 g (82%). FD (M^+): m/z=563.7 g/mol; mp 244°C; ^{1}H NMR (250 MHz, CD_2Cl_2) δ 7.75–7.25 (m, 10H), 7.23–7.11 (m, 15H), 6.69 (d, J=9 Hz, 2H), 6.48 (d, J=9 Hz, 2H).

5.2.2. Compound 2b. 3,4-Bis(4-bromophenyl)-2,5-diphenylcyclopenta-2,4-dien-1-one (**1b**) (5.0 g, 9.22 mmol), BINAP (0.631 g, 1.01 mmol), tris(dibenzylideneacetone)-dipalladium(0) (0.337 g, 0.37 mmol) and $CsCO_3$ (20 g, 0.084 mol) were mixed in a 500 mL flask. The system was flushed with argon and freshly distilled toluene (250 mL) was added. Subsequently, benzophenone imine (20 mL, 0.11 mol) was added dropwise. The reaction mixture was left at 80°C for 4 days. Next, it was cooled down to rt and the toluene was evaporated. The residue was dissolved in tetrahydrofuran and precipitated in a mixture of methanol/water (7/1) to give **2b** as a brown powder. Yield: 6.2 g (83%). FD MS: (M^+): m/z=742.3 g/mol; mp 205°C; ^{1}H NMR (250 MHz, CD_2Cl_2) δ 7.75–7.25 (m, 20H), 7.23–7.11 (m, 10H), 6.69 (d, J=9 Hz, 4H), 6.48 (d, J=9 Hz, 4H).

5.2.3. Compound 5a. Tetra-(4-ethynylphen-1-yl)-methane (0.1 g, 0.24 mmol) and **2a** (0.810 g, 1.44 mmol) in *o*-xylene (10 mL) were heated at 160°C for 24 h under argon atmosphere. Next, the reaction mixture was concentrated to 1/3 of its initial volume and the product was purified by precipitation in a mixture of methanol and water (7/1) to give a dark yellow powder. Yield: 0.520 g (85%). MALDI-TOF MS: m/z=2560.22 g/mol (M^+); ^{1}H NMR (250 MHz, CD_2Cl_2) δ 7.78–6.40 (m, 128H), 6.30–6.15 (m, 8H).

5.2.4. Compound 5b. Tetra-(4-ethynylphen-1-yl)-methane (0.1 g, 0.24 mmol) and **2b** (1.070 g, 1.44 mmol) in *o*-xylene (15 mL) were heated at 160°C for 24 h under argon atmosphere. The reaction mixture was concentrated to 1/4 of the initial volume and the product was purified by precipitation in a mixture of methanol and water (7/1) to give a dark yellow powder. Yield: 0.7 g (89%). MALDI-TOF MS: m/z=3277 g/mol (M^+); ^{1}H NMR (250 MHz, CD_2Cl_2) δ 7.78–6.40 (m, 156H), 6.38–6.15 (m, 16H).

5.2.5. Compound 6a. Compound **5a** (0.100 g, 0.039 mmol) was dissolved in THF (3 mL). Then, 2N HCl (6 mL) was added. After 10 min the mixture was cooled with ice and conc. HCl (3 mL) was added. The mixture was stirred for 20 min. The obtained precipitate was filtered and dried under high vacuum. The final product is a brown powder. Yield: 0.065 g (88%). ^{1}H NMR (250 MHz, DMF-d^6) δ 7.49 (s, 4H), 7.30–6.76 (m, 84H), 6.76–6.60 (m, 8H).

5.2.6. Compound 6b. This was deprotected analogously to **6a**. From **5b** (0.100 g, 0.030 mmol) was obtained 0.050 g (83%) **6b**. ^{1}H NMR (250 MHz, DMF-d^6) δ 7.50 (s, 4H), 7.30–7.11 (m, 48H), 7.09–6.80 (m, 24H), 6.78–6.60 (m 16H).

5.2.7. Compound 7. 4-Maleimide-butyric acid (1.0 g, 5.5 mmol) was dissolved in benzene (10 mL) and thionyl

chloride (1 mL) was added. The reaction mixture was refluxed for 2 h at 70°C. Subsequently, the benzene and unreacted thionyl chloride was evaporated to give **7** as a white powder. Yield: 1.10 g (99%). ^{1}H NMR (250 MHz, CD_2Cl_2) δ 6.73 (s, 2H), 3.58 (t, J=6.7 Hz, 2H), 2.96 (t, J=7.22 Hz, 2H), 1.88 (p, J=6.9 Hz, 2H).

5.2.8. Compound 8a. Compound **6a** (0.065 g, 0.034 mmol) and **7** (0.085 g, 0.41 mmol) were dissolved in a mixture of DMF (5 mL) and triethylamine (0.5 mL). The solution was stirred for 6 h at rt under argon atmosphere. Then the product was purified by precipitation in methanol to give a dark yellow powder. Yield: 0.080 g (92%). MALDI-TOF MS: m/z=2584 g/mol ($M(Na^+)$); ^{1}H NMR (250 MHz, CD_2Cl_2) δ 7.52–6.40 (m, 104H, arom.), 3.53 (m, 8H), 2.19 (m, 8H), 1.90 (m, 8H).

5.2.9. Compound 8b. Compound **6b** (0.050 g, 0.025 mmol) and **7** (0.135 g, 0.61 mmol) were dissolved and stirred in a mixture of DMF (5 mL) and triethylamine (0.5 mL) to give **8b** after precipitation as a dark yellow powder. Yield: 0.072 g (88%). MALDI-TOF MS: m/z=3393 g/mol ($M(Ag^+)$); ^{1}H NMR (250 MHz, DMSO-d^6) δ 7.40–6.40 (m, 108H, arom.), 3.44 (m, 16H), 2.17 (m, 16H), 1.78 (m, 16H).

5.2.10. Compound 9a,b. HOOC-Cys-$(Lys)_5$-NH_2 (**9a**) and HOOC-$(Glu)_5$-Cys-NH_2 (**9b**) were prepared by solid-phase peptide synthesis (SPPS) methods using standard Fmoc-chemistry.[29] The sulfhydryl group of cysteine was protected with a trityl-group, the ε-amino function of lysine with a *tert*(butoxycarbonyl) group (*t*Boc) and the γ-carboxylic acid function of glutamic acid with a *tert*(butyl) group (*t*Bu). A commercially available Wang resin was used, which releases the peptide readily within 1 h after treatment with a cleavage mixture consisting of 94.5% trifluoroacetic acid, 1% triisopropylsilane, 2.5% water and 2% ethanedithiol. The protective groups are simultaneously removed during this procedure and at the C-terminus of the peptide a carboxylic group is generated. Subsequently, the free resin is filtered off and washed with dichloromethane. The solvents are evaporated leaving a small volume for easy precipitation with cold diethylether. The peptide was dried overnight under vacuum. No further purification step was required. Typically, a 1.00 mmol SPPS resulted in 1.2 g (91%) of the peptide as a white powder.

Compound **9a**. ^{1}H NMR (250 MHz, D_2O) δ 4.55 (t, 1H, H_α-Cys, J=5.5 Hz), 4.32–4.20 (m, 4H, H_α-Lys), 4.00–3.95 (t, 1H, N-terminal H_α-Lys, J=6.5 Hz), 2.97–2.95 (10H, H_ε-Lys and 2H, H_β-Cys), 1.88–1.58 (m, 20H, H_β- and H_δ-Lys), 1.50–1.38 (m, 10H, H_γ-Lys).

Compound **9b**. ^{1}H NMR (250 MHz, D_2O) δ 4.47–4.27 (m, 5H, H_α-Glu), 4.21 (t, 1H, H_α-Cys, J=5.5 Hz), 3.18–3.01 (2H, H_β-Cys), 2.60–2.32 (m, 10H, H_γ-Glu), 2.30–1.83 (m, 10H, H_β-Glu).

5.2.11. Compound 11a. Compound **4** (0.060 g, 0.0295 mmol) and **2a** (0.200 g, 0.354 mmol) were dissolved in 9 mL of a tetraethyleneglycol/biphenylether mixture (1/1). The resulting solution was heated for 24 h at 170°C under argon atmosphere. The reaction mixture was precipitated in methanol to give **11a** as a dark yellow powder. Yield: 0.17 g (91%). MALDI-TOF MS: m/z=6319 g/mol; ^{1}H NMR (250 MHz, CD_2Cl_2) δ 7.78–6.40 (m, 316H), 6.30–6.15 (m, 16H).

5.2.12. Compound 11b. Compound **4** (0.060 g, 0.0295 mmol) and **2b** (0.354 mmol) were dissolved in 9 mL of a tetraethyleneglycol/biphenylether mixture (1/1). The resulting solution was heated for 24 h at 170°C under argon atmosphere. The reaction mixture was precipitated in methanol to give **11b** as a dark yellow powder. Yield: 0.205 g (89%). MALDI-TOF MS: m/z=7753 g/mol (M^+); ^{1}H NMR (250 MHz, CD_2Cl_2) δ 7.78–6.40 (m, 372H), 6.37–6.15 (m, 32H).

5.2.13. Compound 12a,b. These were deprotected analogously to **5a**. From **11a** (0.120 g, 0.019 mmol), **12a** (0.075 g, 80%) was obtained and from **11b** (0.120 g, 0.015 mmol), **12b** (0.065 g, 84%) was obtained.

Compound **12a**. ^{1}H NMR (250 MHz, DMF-d^6) δ 7.50–6.75 (m, 236H), 6.74–6.58 (m, 16H).

Compound **12b**. ^{1}H NMR (250 MHz, DMF-d^6) δ 7.50–6.76 (m, 212H), 6.75–6.58 (m, 32H).

5.2.14. Compound 13a. This was prepared analogously to **8a**. **12a** (0.065 g, 0.013 mmol) and **7** (0.139 g, 0.63 mmol) were dissolved and stirred in a mixture of DMF (5 mL) and triethylamine (0.5 mL) to give **13a** as a dark yellow powder. Yield: 0.075 g (91%). MALDI-TOF MS: m/z=6435 g/mol $M(Ag^+)$) (broad peak); ^{1}H NMR (250 MHz, DMF-d^6) δ 7.50–6.40 (m, 268H, arom.), 3.47 (m, 16H), 2.25 (m, 16H), 1.81 (m, 16H).

5.2.15. Compound 13b. This was prepared analogously to **8a**. **12b** (0.065 g, 0.013 mmol) and **11** (0.139 g, 0.63 mmol) were dissolved and stirred in a mixture of DMF (5 mL) and triethylamine (0.5 mL) to give **13b** as a dark yellow powder. Yield: 0.095 g (94%). MALDI-TOF MS: m/z=7770 g/mol (broad peak); ^{1}H NMR (250 MHz, DMF-d^6) δ 7.50–6.40 (m, 276H, arom.), 3.47 (m, 32H), 2.25 (m, 32H), 1.81 (m, 32H).

5.2.16. Compound 10a–c and 14b. 1 equiv. of the corresponding maleimide-decorated dendrimer and 3 equiv. of **9a** or **9b** were dissolved in DMF (10 mL). The reaction mixture was stirred for 72 h at rt. DMF was removed by dialysis against distilled water for 48 h. Finally, the peptide-decorated dendrimers were purified by RP-HPLC.

Compound **10a**. Yield: 80%. ^{1}H NMR (500 MHz, DMSO-d^6) δ 7.55–6.40 (m, 96H, arom.), 4.49–4.20 (m, 20H), 4.01 (m, 4H), 3.79 (m, 4H), 3.49 (m, 8H), 3.25 (m, 8H), 3.05 (m, 8H), 2.80 (m, 40H), 2.22 (m, 8H) 1.89–1.60 (m, 88H), 1.51–1.38 (m, 40H); RP-HPLC: retention time 33 min.

Compound **10b**. Yield: 65%. ^{1}H NMR (500 MHz, DMSO-d^6) δ 7.55–6.40 (m, 92H, arom.), 4.49–4.20 (m, 40H), 3.99 (m, 8H), 3.81 (m, 8H), 3.48 (m, 16H), 3.25 (m, 16H), 2.97 (m, 16H), 2.80 (m, 80H), 2.22 (m, 16H) 1.89–1.60 (m, 176H), 1.51–1.38 (m, 80H); RP-HPLC: retention time 47 min.

Compound **10c**. Yield: 62%. ^{1}H NMR (500 MHz, DMSO-d^6) δ 7.55–6.40 (m, 92H, arom.), 4.22–4.10 (m, 40H), 4.01 (m, 8H), 3.79 (m, 8H), 3.49 (m, 16H), 3.25 (m, 16H), 3.05 (m, 16H), 2.40–2.15 (m, 96H), 1.89–1.70 (m, 96H); RP-HPLC: retention time 10 min.

Compound **14a**. Yield: 39%. ^{1}H NMR (500 MHz, DMF-d^6) δ 7.65–6.40 (m, 252H, arom.), 4.65–4.35 (m, 40H), 4.20–4.00 (m, broad, 32H), 3.65–3.45 (m, 16H), 3.25 (m, 16H), 2.89–3.10 (m, broad, 96H), 2.38–2.50 (m, broad, 16H) 1.95 (m, 16H), 1.88–1.60 (m, 160H), 1.50–1.38 (m, 80H); RP-HPLC: retention time 69 min.

Compound **14b**. Yield: 45%. ^{1}H NMR (500 MHz, DMF-d^6) δ 7.65–6.40 (m, 244H, arom.), 4.65–4.35 (m, 80H), 4.21–4.00 (m, broad, 64H), 3.60–3.40 (m, 32H), 3.25 (m, 32H), 2.89–3.10 (m, broad, 192H), 2.38–2.50 (m, broad, 32H) 1.95 (m, 32H), 1.88–1.60 (m, 320H), 1.51–1.38 (m, 160H). RP-HPLC: retention time 58 min.

5.2.17. Compound 15a. This was obtained analogously to **6a** by deprotection of **2a** (1.500 g, 2.66 mmol) to give **15a** as a red powder. Yield: 1.02 g (96%). FD (M^+): m/z=399.6 g/mol; ^{1}H NMR (250 MHz, DMF-d^6) δ 7.30–7.16 (m, 15H), 6.90–6.79 (m, 4H).

5.2.18. Compound 15b. This was obtained analogously to **6a** by deprotection of **2b** (1.800 g, 2.42 mmol) to give **15b** as a red powder. Yield: 0.95 g (94%). FD (M^+): m/z=414.5 g/mol; ^{1}H NMR (250 MHz, DMF-d^6) δ 7.32–7.16 (m, 10H), 6.90–6.79 (m, 4H).

5.2.19. Compound 17a. This was prepared from **15a** (0.799 g, 2 mmol), which was added to N^{α},N^{ε}-*Z*-Lys (2.484 g, 6 mmol), EDC (1.152 g, 6 mmol) and DMAP (0.406 g, 3.33 mmol) in DMF (10 mL), and stirred for 72 h under argon atmosphere. Subsequently, DCM (10 mL) was added and the reaction mixture was washed twice with water. The organic phase was isolated, dried on magnesium sulfate and subsequently the solvent was evaporated. The dried residue was purified by column chromatography with DCM/ethylacetate (5/1) as eluent to give **17a** as a red powder. Yield: 1.25 g (79%). FD (M^+): m/z=796 g/mol; ^{1}H NMR (250 MHz, CD_2Cl_2) δ 7.55–7.10 (m, 25H, arom.), 6.96 (d, 2H, J=7.90 Hz), 6.85 (d, 2H, J=7.90 Hz), 5.1 (s, 4H), 4.15 (t, 1H), 3.16 (m, 2H), 1.89–1.60 (m, 4H), 1.51–1.38 (m, 2H).

5.2.20. Compound 17b. This was prepared from **15b** (0.829 g, 2 mmol), which was added to N^{α},N^{ε}-*Z*-Lys (4.968 g, 12 mmol), EDC (2.304 g, 12 mmol) and DMAP (0.812 g, 6.66 mmol) in DMF (15 mL). The solution was stirred for 72 h under argon atmosphere. Subsequently, DCM (10 mL) was added and the reaction mixture was washed twice with water. The organic phase was dried on magnesium sulfate and the solvent evaporated. The dried residue was purified by column chromatography with DCM/ethylacetate (3/1) as eluent to give **17a** as red powder. Yield: 1.10 g (45%). FD (M^+): m/z=1208 g/mol; ^{1}H NMR (250 MHz, CD_2Cl_2) δ 7.55–7.10 (m, 34H, arom.), 6.81 (d, 4H, J=7.85 Hz), 5.06 (m, 8H), 4.18 (t, 2H), 3.14 (m, 4H), 1.89–1.60 (m, 8H), 1.51–1.38 (m, 4H).

5.2.21. Compound 18. This was prepared analogously to **11a** from tetra-(4-ethynylphen-1-yl)-methane (0.05 g, 0.12 mmol) and **17a** (0.460 g, 0.58 mmol) to give **18** as a yellow powder. Yield: 0.40 g (95%). MALDI-TOF MS: m/z=3511 g/mol ($M(Na^+)$); ^{1}H NMR (250 MHz, CD_2Cl_2) δ 7.55–6.40 (m, 136H, arom.), 5.05 (m, 16H), 4.06 (m, 4H), 3.08 (m, 8H), 1.89–1.60 (m, 16H), 1.51–1.38 (m, 8H).

5.2.22. Compound 20a. This was prepared analogously to **11a** from **4** (0.060 g, 0.0295 mmol) and **17a** (0.263 g, 0.354 mmol) to give **20a** as a yellow powder. Yield: 0.21 g (87%). MALDI-TOF MS: m/z=8198 g/mol ($M(Na^+)$); ^{1}H NMR (250 MHz, CD_2Cl_2) δ 7.55–6.40 (m, 332H, arom.), 5.05 (m, 32H), 4.06 (m, 8H), 3.08 (m, 16H), 1.89–1.60 (m, 32H), 1.51–1.38 (m, 16H).

5.2.23. Compound 20b. This was prepared analogously to **11a** from **4** (0.060 g, 0.0295 mmol) and **17b** (0.427 g, 0.354 mmol) to give **20b** as a yellow powder. Yield: 0.30 g (89%). ^{1}H NMR (250 MHz, d^2-DCM): 7.55–6.40 (m, 404H, arom.), 5.05 (m, 64H), 4.06 (m, 16H), 3.08 (m, 32H), 1.89–1.60 (m, 64H), 1.51–1.38 (m, 32H).

5.2.24. Compound 19 and 21a,b. These were prepared by deprotection of the **18** and **20a**,**b**. To 0.100 g of each dendrimer was dissolved 1 mL 10% solution of HBr in ice-acetic acid. The mixture was stirred for 1 h at rt and precipitated in diethylether to give deprotected Lys-decorated dendrimers.

Compound **19**. Yield: 0.065 g (95%). MALDI-TOF MS: m/z=2415 g/mol (M^+); ^{1}H NMR (250 MHz, DMF-d^6) δ 7.40–6.40 (m, 96H, arom.), 4.38 (m, 4H), 2.94 (m, 8H), 1.95–1.60 (m, 16H), 1.51–1.38 (m, 8H).

Compound **21a**. Yield: 0.07 g (96%). ^{1}H NMR (250 MHz, DMF-d^6) δ 7.55–6.40 (m, 252H, arom.), 4.42 (m, 8H), 2.90 (m, 16H), 1.89–1.60 (m, 32H), 1.51–1.38 (m, 16H).

Compound **21b**. Yield: 0.06 g (96%).^{1}H NMR (250 MHz, DMF-d^6) δ 7.55–6.40 (m, 244H, arom.), 4.44 (m, 16H), 2.90 (m, 32H), 1.88–1.60 (m, 64H), 1.51–1.38 (m, 32H),

Acknowledgements

Financial support from the Deutsche Forschungsgemeinschaft within the Sonderforschungsbereich 625 and the Emmy Noether Program (KL 1049/2, H. -A. K.), the Stiftung Stipendien-Fonds des Verbandes der Chemischen Industrie and the Bundesministerium für Bildung und Forschung (G. V.) is gratefully acknowledged.

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